The growth of hydroxyapatite on alkaline treated Ti–6Al–4V soaking in higher temperature with concentrated $\text{Ca}^{2+}/\text{HPO}_4^{2-}$ simulated body fluid

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Abstract

In this study, calcium and phosphorous ions in the simulated body fluid (SBF) was be increased to increase the rate of precipitation of hydroxyapatite (HA). The soaking temperature in concentrated calcium and phosphorous ion-SBF (CP-SBF) was increased to reduce the nucleation energy of the HA, which lead to an early precipitation to shorten the treatment process. When the metallic substrates treated with 10 M NaOH aqueous solution and subsequently heated at 600 °C, a thin sodium titanium oxide layer was formed on the surfaces as the linking layer for HA and Ti–6Al–4V alloys. After Ti–6Al–4V alloys treated with alkali solution, it would soak into a simulated body fluid with higher concentration of calcium and phosphorous ions (CP-SBF) to increase the possibility of nucleation of HA. When Ti–6Al–4V alloys treated with alkali solution, subsequently heated at 600 °C, and then soaked into CP-SBF at a temperature of 80 °C, it could form a dense and thick (50 μm) bone-like hydroxyapatite layer on the surface. The HA layer was appeared on the surface of the Ti-alloy at the first week soaking, which was greatly shorten the coating process. In the research, the characteristics of the coating layer will be analyzed by the results of X-ray diffractometer (XRD), scanning electron microscope (SEM), and Fourier transformation infrared (FT-IR).

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1. Introduction

Generally, artificial materials implanted into bone defects are encapsulated by fibrous tissue isolated from the surrounding bone. Implant materials to be used as substitutes for high load-bearing bones, such as femoral and tibia bones, need to possess not only bone-bonding ability but also high fracture toughness [1]. However, neither the currently available bioactive ceramics nor a biocompatible metal fulfills these requirements. The fracture toughness of the ceramics is lower than that of human cortical bone, and none of the metals directly bonds to living bone. Titanium and titanium alloys are widely used in orthopedic implants because of their superior mechanical properties with a tensile strength of 860 MPa and yield strength of 775 MPa, and its lightness with a specific gravity of 4.5 g cm⁻³ [2]. However, Ti-based

alloys are not the best from a biological point of view since they may corrode away when implanted. Calcium phosphate (Ca–P) apatite has been well known to be suitable for bone repair, augmentation, substitution, and surface coating [3]. Coating dental and orthopedic materials with biocompatible calcium phosphate apatite enable one to mimic the reactions occurring in the natural calcified tissues without losing bulk properties of materials [4–7].

The hydroxyapatite (HA) has excellent bioactivity but unsatisfactory in mechanical properties, with a bending strength less than 100 MPa. The coating of Ti alloy with HA give rise to a favorable combination of the good mechanical properties of Ti-based alloys with the good bio-behavior of HA. The coating on Ti metal or Ti alloys has been produced by pulsed laser deposition [8], ion-beam deposition [9] or sol-gel [10]. Currently, one of the most common practices for actual clinical application is by plasma spray of HA onto Ti alloys at an elevated temperature near 10,000 °C. Nevertheless, the produced coating is a mechanically bonded HA layer and is liable to peel off [11–17]. The degradation of

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the coated HA layer is inevitable due its thermal unstability [18].

Recently, in vitro studies showed that titanium hydrogel prepared by a sol–gel process or by alkali and heat treatments could induce bone-like apatite on titanium substrate in simulated body fluid (SBF) [19–24]. However, the bioactive layer produced by this method could be only a relatively thin film [1].

This present study increased the calcium and phosphorous ion concentration in the SBF (CP-SBF) and increased the soaking temperature to increase the thickness of the HA coating layer on alkali treated Ti–6Al–4V alloys. Thus produced bioactive layer were characterized with scanning electron microscope (SEM), X-ray diffractometer (XRD) and Fourier transformation infrared (FT-IR).

2. Materials and methods

2.1. Specimen and surface preparation

Ti–6Al–4V plate was supplied by the Materials Research Laboratories, Industrial Technology Research Institute (ITRI, Hsinchu, Taiwan). The titanium alloy with 10 mm × 10 mm × 1 mm in dimension were abraded with #320, #400, #600, #1000 SiC sequentially, and cleaned with acetone and distilled water in an ultrasonic cleaner. Alkali treatment was performed by soaking thus prepared substrates in 5 mL of 10 M NaOH aqueous solution at 60 °C for 24 h [25–29]. After the alkali treatment, the substrates were gently washed with distilled water and dried at 40 °C for 24 h in an air atmosphere. The alkali-treated substrates were then heated to 500–800 °C at a rate of 5 °C min^{−1} in an Ni–Cr electric furnace, held at a given temperature for 1 h, and then cooled to room temperature in the furnace.

2.2. Soaking in simulated body fluid

The simulated body fluid (SBF) applied exhibits ion concentrations nearly equal to those of human blood plasma, as shown in Table 1 [30,31]. The CP-SBF in Table 1 was a modified SBF with higher concentration product of Ca²⁺ and PO₄^{3−}. The two simulated body fluids were prepared by dissolving reagent grade NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, and Na₂SO₄ in distilled water, and buffered at pH 7.40 with hydrochloric acid and tris-hydroxymethylaminomethane [(CH₂OH)₃CNH₂] at

37 °C. Each specimen was immersed in different conditions of solution as shown in Table 2. The treated specimens were washed gently with pure acetone and dried in a clean bench.

2.3. Analysis of surface and solution

The surface structural change of the tested specimens was examined by X-ray diffractometer (XRD) using Cu Kα radiation with a wavelength of 0.154 nm and operated at the condition of 40 kV and 20 mA. The goniometer was set at a scan rate of 4° min^{−1} with 2θ range of 10–60°. Scanning electron microscopy (SEM) was used for morphological observation and infrared spectroscopy (IR) was used for chemical analysis. The infrared (IR) spectrum was set with a resolution of 8 cm^{−1} within the range of 400–4000 cm^{−1}.

3. Results

Fig. 1 showed the XRD patterns of the Ti–6Al–4V alloy substrate treated with 10 M NaOH at 60 °C for 24 h and heated to various temperatures. Fig. 1a shows the typical XRD pattern of Ti-alloy substrate. The alkali solution treatment did not change the diffraction pattern (Fig. 1b) as expected. After treated with alkali solution and subsequent heat treatment at 500 °C, the pattern shows only peaks corresponding to Ti-alloy (Fig. 1c). A sodium titanium oxide (Na₂Ti₅O₁₁) was formed on the surface after subsequent heat treatment at the temperature of 600 °C (Fig. 1d). Once the temperature up to 700 °C, an unknown phase was appeared on the pattern which could not be identified from the current JCPD file. Sodium titanium oxide (Na₂Ti₅O₁₁) still appeared at the temperature of 700 °C as shown in Fig. 1e. Fig. 1f was the XRD pattern of Ti-alloy treated with alkali solution and then heated at 800 °C. It showed a thick layer of rutile covered on the surface of the substrate. There were no peaks belonging to Ti-alloy or any other phases appeared on the pattern. An inter-compound plays an important role to link up the Ti-alloy substrate and the later coated HA layer. The 600 °C was thought to be the optimum temperature for heat treatment and would be used for the later experiments.

Fig. 2 shows the XRD pattern of Ti-alloy treated with Al condition of Table 2, where previous treated Ti-alloys was soaked in CP-SBF at the temperature of 37 °C for 1–4 weeks. Hydroxyapatite (HA) was not detected when soaked upto 3 weeks (Fig. 2a–c). HA appeared when soaked for 4 weeks (Fig. 3d). The broadening peaks were due to the

Table 1

Ionic concentrations of SBF and CP-SBF solutions, in comparison with those of human blood plasma

	Concentration (mM)							
	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl [−]	HCO ₃ [−]	HPO ₄ ^{2−}	SO ₄ ^{2−}
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	27.0	0.5
SBF	142.0	5.0	1.5	2.5	147.8	4.2	4.2	0.5
CP-SBF	142.0	5.0	1.5	3.75	147.8	4.2	4.2	0.5

Fig. 3. XRD patterns of Ti-6Al-4V alloys treated as A2 condition of Table 2: (a) 1 week, (b) 2 weeks, (c) 3 weeks, (d) 4 weeks.

and 1490 cm^{-1} . It reflected that carbonate ions was incorporated in the HA structure as a biological apatite.

4. Discussion

As mentioned earlier, the requirement for titanium to bond to living bone is for the formation of a biologically active bone-like apatite on their surface. It has been confirmed that the formation of this apatite on the surfaces of materials in vivo can be produced in SBF [22]. In view of these facts, the titanium and its alloys treated by the method of previous studies are believed to form the bone-like apatite layer on their surfaces. However, the reported methods could only form a thin film of HA on the surface and was not durable for long-term application.

The Ostwald's nucleation theory [35] describes that the free energy for nucleation depends on the supersaturation of solution (S), the net interfacial energy for nucleation (σ), the temperature (T), and the particle surface area (A):

$$\Delta G = -RT(\ln S) + \sigma A$$

Fig. 4. XRD patterns of Ti-6Al-4V alloys treated as A3 condition of Table 2: (a) 1 week, (b) 2 weeks, (c) 3 weeks, (d) 4 weeks.

Basing on this heterogeneous nucleated by nucleation theory, it is possible to induce increasing the solution supersaturation S and reducing the net interfacial energy σ . In the present study, the solution supersaturation S was greatly increased by increasing the product of Ca^{2+} and HPO_4^{2-} in SBF (CP-SBF) to reduce the free energy for nucleation (ΔG). Homogeneous precipitation occurring in highly supersaturated solutions was prevented by using a low temperature system. In this study, the temperature for HA precipitation was also increased to 80°C to reduce the nucleation free energy of the HA coating.

When the Ti-alloy was treated with A1 condition, the HA appeared at 4-week soaking due to higher nucleation energy in lower precipitation temperature of 37°C (Fig. 2). If Ti-alloy treated with A3 condition, the substrate was soaked in SBF at the temperature of 80°C . Though increased the soaking temperature, the concentration of calcium and phosphorous ions was relatively low in SBF. The HA coating layer appeared at 2-week soaking (Fig. 3). However, the thickness of the HA layer was only around $10\text{--}15\text{ }\mu\text{m}$ (Fig. 6). If increase the production of $\text{Ca}^{2+}/\text{HPO}_4^{2-}$ in SBF (CP-SBF) and increase the soaking temperature, the

Fig. 5. SEM photographs of Ti-6Al-4V alloys: (a) after alkali treatment and (b) after subsequent heat treatment at 600 °C for 1 h.

nucleation energy decrease in great amplitude which cause to the HA layer to precipitate on surface earlier (Fig. 4) with a thicker coating around 40–50 μm (Fig. 6).

The different conditions immersion results in this work indicate that all of alkali treatment can produce the titanium surface allowing the Ca-P crystal growth. The effect of immersion temperature and concentration could increase the coating thickness. The alkali treatment is necessary to acquire a new TiO_2 surface layer on titanium instead of the naturally formed one because the OH^- groups, so we

immersed titanium alloy in 10 M alkali solution to produce an active hydrogel surface layer that was subsequently treated at 600 °C to stabilize it compared as $\beta\text{-TiO}_2$ crystalline at 700 °C, which are always on TiO_2 surface at aqueous environment, could benefit the induction of Ca-P from different conditions of solution [32]. To understand the accurate relationship between Ca-P indication and titanium surface properties, the detailed nucleation process of Ca-P on the chemically treated titanium surface had been investigated [33].

Fig. 6. The SEM micrographs of the HA precipitation as the conditions of: (a) A1, (b) A2, (c) A3, soaked for 4 weeks.

A biologically apatite with carbonate containing can be obtained by the present method. Vignoles, Bonel, and Montel in [34] have reported that carbonate-containing calcium-deficient HA could be precipitate at higher pH value of 8.5. As shown in Fig. 7, the absorption bands for carbonate and HPO_4^{2-} could be observed on the spectra. It proved that a thick layer of biological hydroxyapatite could be precipitated on the surface of the Ti-alloy after treated in alkali solution at 60°C , subsequent heat treatment at 600°C , and then soaked in CP-SBF solution at the temperature of 80°C for 4 weeks.

Fig. 7. FT-IR absorbance spectra of the Ti-alloy after alkali treated, subsequent heat treatment at 600°C and then immersed in CP-SBF at 80°C for 4 weeks.

5. Conclusions

A thick biological HA layer was successfully coated on the Ti-alloy after treatment by soaking in CP-SBF at 80°C followed by heat treatment at 600°C . Increasing the production of calcium and phosphorous ions in SBF and increasing the soaking temperature could effectively reduce the nucleation energy of the hydroxyapatite, that could lead to rapid precipitation of HA. This also gave rise to the behavior of precipitation at relatively lower temperature. The method described herein has a great potential to the future application on the surface treatment of metallic substrate before used in vivo.

References

- [1] H.M. Kim, F. Miyaji, T. Kokubo, S. Mishighchi, T. Nakamura, Graded surface structure of bioactive titanium prepared by chemical treatment, *J. Biomed. Mater. Res.* 45 (1999) 100–107.
- [2] J.B. Park, R.S. Lakes, *Biomaterials An Introduction*, Plenum Press, New York, 1992.
- [3] G.M. Barrow, *Physical Chemistry*, sixth ed., McGraw-Hill, 1996.
- [4] J.D. de Bruijn, C.A. van Blitterswijk, J.E. Davies, Initial bone matrix formation at the hydroxyapatite interface in vitro, *J. Biomed. Mater. Res.* 29 (1) (1995) 89–99.
- [5] M. Jarcho, Calcium phosphate ceramics as hard tissue prosthetics, *Clin. Orthop.* 157 (1995) 259–278.

- [6] M. Jarcho, Retrospective analysis of hydroxyapatite development for oral implant applications, *Dent. Clin. North Am.* 36 (1) (1992) 19–26.
- [7] M. Okumura, H. Ohgushi, Y. Dohi, T. Katuda, S. Tamai, H.K. Koerten, S. Tabata, Osteoblastic phenotype expression on the surface of hydroxyapatite ceramics, *J. Biomed. Mater. Res.* 37 (1997) 122–129.
- [8] C.K. Eang, J.H. Chern Lin, C.P. Ju, Structural characterization of pulsed laser-deposited hydroxyapatite film on titanium substrate, *Biomaterials* 18 (1997) 1331–1338.
- [9] J.L. Ong, L.C. Lucas, W.R. Lacefield, E.D. Rigney, Structure, solubility and bond strength of thin calcium phosphate coatings produced by ion beam sputter deposition, *Biomaterials* 13 (1992) 249–254.
- [10] P. Li, K. de Groot, Calcium phosphate formation within sol–gel prepared titania in vitro and in vivo, *J. Biomed. Mater. Res.* 27 (1993) 1495–1500.
- [11] K.A. Tomas, J.F. Kay, S.D. Cook, M. Jarcho, The effect of surface macrotexture and hydroxyapatite coating on the mechanical strength and histological profiles of titanium implant material, *J. Biomed. Mater. Res.* 21 (1987) 1395–1406.
- [12] W.R. Lacefield, Hydroxyapatite coatings, in: P. Ducheyne, J. Lemons (Eds.), *Bioceramics: Material Characteristics versus In Vitro Behavior*, Academy of Sciences, New York, 1998, pp. 72–80.
- [13] M.J. Filiaggi, R.M. Pilliar, Interfacial characterization of plasma sprayed hydroxyapatite/Ti–6Al–4V implant system, in: *Annual Conference of the Canadian Biomaterial Society on Transactions of ABCS*, 1989, pp. 23–25.
- [14] S. Radin, P. Ducheyne, The effect of plasma sprayed induced changes in the characteristics on the in vitro stability of calcium phosphate ceramic, *J. Mater. Sci.* 3 (1992) 33–42.
- [15] C.P.A.T. Klein, J.G.C. Wolke, J.M.A. de Bleeck-Hogewerst, K. de Groot, Features of calcium phosphate plasma-sprayed coatings: An in vitro study, *J. Biomed. Mater. Res.* 28 (1994) 961–967.
- [16] K.A. Gross, C.C. Berndt, In vitro testing of plasma sprayed hydroxyapatite coating, *J. Mater. Sci. Mater. Med.* 5 (1994) 219–224.
- [17] K.A. Mann, A.A. Edidin, R.K. Kinoshita, M.T. Manley, Mixed mode fracture characterization of hydroxyapatite–titanium alloy interface, *J. Appl. Biomater.* 5 (1994) 285–291.
- [18] A. Ravaglioli, A. Krajewski, *Bioceramics: Materials, Properties, Applications*, Chapman and Hall Press, London, 1992.
- [19] H.B. Wen, J.R. de Wijn, F.Z. Cui, K. de Groot, Preparation of bioactive Ti6Al4V surfaces by a simple method, *Biomaterials* 19 (1998) 215–221.
- [20] M.C. de Andrade, M.R.T. Filgueiras, T. Ogasawara, “Nucleation and growth of hydroxyapatite on titanium pretreated in NaOH solution” Experiments and thermodynamic explanation”, *J. Biomed. Mater. Res.*, 46 (1999) 441–446.
- [21] B.C. Yang, J. Weng, X.D. Li, X.D. Zhang, The order of calcium and phosphate ion deposition on chemically treated titanium surfaces soaked in aqueous solution, *J. Biomed. Mater. Res.* 47 (1999) 213–219.
- [22] S. Nishiguchi, T. Nakamura, M. Kobayashi, H.M. Kim, F. Miyaji, T. Kokubo, The effect of heat treatment on bone-bonding ability of alkali-treated titanium, *Biomaterials* 20 (1999) 491–500.
- [23] R. Viitala, J. Simola, T. Peltola, H. Rahiala, M. Linden, M. Langlet, J.B. Rosenholm, In vitro bioactivity of aerosol-gel deposited TiO₂ thin coatings, *J. Biomed. Mater. Res.* 54 (2001) 109–114.
- [24] K. Sato, Y. Kumagai, J. Tanaka, Apatite formation on organic monolayers in simulated body environment, *J. Biomed. Mater. Res.* 50 (2000) 16–20.
- [25] F.H. Lin, W.H. Wang, C.P. Lin, Transition element contained PSA as a dental retrograde-filling materials, *Biomaterials* 24 (2003) 219–233.
- [26] H.F. Lin, Y.S. Hsu, S.H. Lin, J.S. Sun, The effect of Ca/P concentration and temperature of SBF on the growth of HAP coating on 316L SS, *Biomaterials* 23 (2002) 4029–4038.
- [27] L.D. Arsov, C. Kormann, W. Plieth, In situ Raman spectra of anodically formed titanium dioxide layer in solution of H₂SO₄, KOH, and HNO₃, *Electrochem. Soc.* 138 (1991) 2964–2970.
- [28] A.R. Prusi, L.D. Arsov, The growth kinetics and optical properties of films formed under open circuit conditions on a titanium surface in potassium hydroxide solution, *Corros. Sci.* 33 (1994) 153–164.
- [29] T. Kokubo, Bioactivity of glasses and glass–ceramics, in: P. Ducheyne, T. Kokubo, C.A. Blitterswijk (Eds.), *Handbook of Bioactive Ceramics*, vol. 1, Bone-Bonding Biomaterials, Reed Healthcare Communications, Leiden, The Netherlands, 1992, pp. 31–46.
- [30] T. Kokubo, Bioactivity of glasses and glass–ceramics, in: P. Ducheyne, T. Kokubo, C.A. Blitterswijk (Eds.), *Handbook of Bioactive Ceramics*, vol. 1, Bone-Bonding Biomaterials, Reed Healthcare Communications, Leiden, The Netherlands, 1992, pp. 31–46.
- [31] T. Kokubo, S. Ito, Z.T. Huang, T. Hatashi, S. Sakka, T. Kitsugi, T. Yamamuro, Ca, P-rich layer formed on high-strength bioactive glass–ceramic A-W, *J. Biomed. Mater. Res.* 24 (1990) 331–343.
- [32] H.M. Kim, F. Miyaji, T. Kokubo, T. Nakamura, Preparation of bioactive Ti and its alloys via simple chemical surface treatment, *J. Biomed. Mater. Res.* 32 (1996) 409–417.
- [33] C. Liu, Y. Huang, W. Shen, J. Cui, Kinetics of hydroxyapatite precipitation at pH 10 to 11, *Biomaterials* 22 (2001) 301–306.
- [34] K. de Groot, *Bioceramics of Calcium Phosphate*, CRC Press Inc., Boca Raton, FL, 1983.
- [35] H.M. Kim, Y. Kim, S.J. Park, C. Rey, H.M. Lee, M.J. Glimcher, J.S. Ko, Thin film of low-crystalline calcium phosphate apatite formed at low temperature, *Biomaterials* 21 (2000) 1129–1134.